In the matter of
US Patent Application
Serial No. 725206
filed April 19th, 1985.

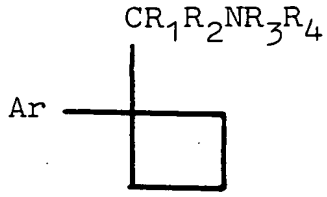
#14

DECLARATION

I, William Roger Buckett, a British Subject of 27 Trevor Road, West Bridgford, Nottingham, England, do hereby declare that:-

1) In 1959 I was awarded the degree of B. Pharm. with Honours in Pharmacology by the University of London, England. In 1965 I was awarded the degree of Ph.D. in Pharmacology from the University of London. In 1983 the same university awarded me a higher doctorate, namely the D.Sc. degree, on the basis of my published work in diverse areas of neuropharmacology. In addition I have been designated a Fellow of the Institute of Biology (F.I. Biol.) and of the Pharmaceutical Society of Great Britain (F.P.S.). From 1960 to date I have been employed in industrial pharmacology in several companies including Organon Laboratories Ltd., and ICI PLC, Pharmaceuticals Division, (United Kingdom), Novo Industri A/S (Denmark) and Centre de Recherche Merrell International (France). In 1980 I joined the Research Department of The Boots Company PLC, Nottingham, England as Section Leader (Mental Illness, Biology). In 1982 I was appointed Research Project Leader (Mental Illness) and in 1984 became Senior Section Leader (Mental Illness Projects). I am in charge of a team of scientists engaged in neuroscience research and am project leader for antidepressant, neuroleptic and antiepileptic projects. I have published over 75 papers and abstracts in scientific journals and am entirely familiar with the pharmacological and biochemical methods used to evaluate antidepressant drug activity in chemical compounds.

2) I am familiar with the compounds described in US Patent 4443449 and in the above identified application which I have been advised by Counsel is a continuation of U.S. Serial No. 365285 (now issued as U.S. Patent No. 4522828) with which I am also familiar. I have been advised by Counsel that US Serial No. 725206 will be amended by an Amendment to be submitted herewith and will after amendment claim compounds of formula B

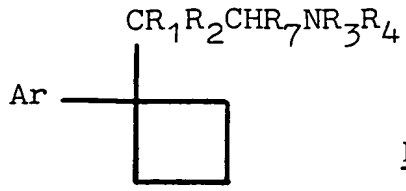


Formula B

in which R_1 is a branched chain alkyl of up to 6 carbon atoms. I am also advised by Counsel that there are other copending applications which describe compounds of formula B. Counsel advises me that US Serial No. 725129 describes and claims compounds of formula II in which R_1 is methyl or ethyl and that US Serial No. 812730 describes and claims compounds of formula B in which R_1 is propyl or butyl. I am advised by Counsel that a divisional application derived from US Serial No. 725206 is to be filed relating to compounds of formula B in which R_1 is cycloalkyl, cycloalkylalkyl, optionally substituted phenyl, alkenyl or alkynyl.

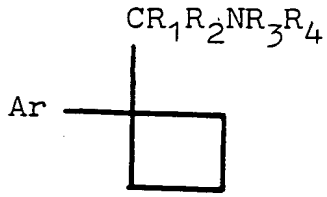
3) I am also familiar with the test procedures conducted at The Boots Company PLC to determine the therapeutic activity of compounds described and claimed in US Patent No. 4443449 and described and claimed in US Serial No. 725206. The reserpine reversal test was conducted in the following manner. Male mice of Charles River CD1 strain weighing between 18 and 30 g were separated into groups of five and were supplied with food and water ad libitum. After four

hours, the body temperature of each mouse was taken orally and the mice were injected intraperitoneally with reserpine (10 mg/kg) in solution in deionised water containing ascorbic acid (50 mg/ml). The amount of liquid injected was 10 ml/kg of body weight. Twenty-three hours after the start of the test the temperatures of the mice were taken and the mice were given the test compound orally at a dose volume of 10 ml/kg of body weight. The compound was administered in one of the following ways:- (a) in aqueous solution, (b) in solution in less than 1% v/v acetic acid, (c) in solution in less than 0.02N hydrochloric acid, (d) suspended in an acacia suspension containing 100 mg acacia in 5 ml deionised water or (e) suspended in a 0.25% solution of hydroxy ethyl cellulose (sold under the trade name Cellosize QP 15000 by Union Carbide) in deionised water. Three hours later the temperatures of all the mice were again taken. The percentage reversal of the reserpine-induced loss of body temperature was then calculated for the 30 mg/kg dosage and is given in the attached table as a percentage reversal (RR_{30}). This test is a meaningful measurement of the antidepressant activity of the compounds tested. According to the procedure at The Boots Company PLC (the assignee of the present application) all compounds submitted for screening are tested at least once at a dose of 30 mg/kg. Many compounds gave a percentage reversal at this dose of greater than 50% and were subsequently tested at lower doses to enable a value of mean dose which would cause a 50% reversal (ED_{50}) to be obtained. In the Tables appended to this Declaration the percentage reversal results at 30 mg/kg is given in the column headed RR_{30}. It is believed that any compound which shows greater than 50% reversal at 30 mg/kg has utility as an antidepressant. In the Tables set forth hereinafter compounds which are described and claimed in US Patent No. 4443449 are represented by formula A



Formula A

and compounds which are described and claimed in US Serial Nos. 725129, 725206 and 812730 are represented by formula B



Formula B

4) During research in the laboratories of the assignee of the present application to find compounds having utility as antidepressants many compounds of formula A and formula B have been synthesised and tested in the test described above. The results obtained in the screening of these compounds has been provided to me and I have reviewed these results which are set forth in the attached Tables.

5) In Table IA attached hereto are given the RR_{30} values determined for each of the compounds identified in the Examples of US Patent 4443449 and in Table IB are given the RR_{30} values for other compounds of formula A falling within the scope of the claims of US Patent 4443449 which have been prepared in the laboratories of the assignees of the present invention.

6) In Tables II and III attached hereto the RR_{30} for compounds of formula A in which Ar is 4-chlorophenyl and 3,4-dichlorophenyl respectively which have been synthesised

in the laboratories of the assignee of the present application are set forth. In addition those Tables also include RR_{30} data on compounds of formula B in which Ar is 4-chlorophenyl and 3,4-dichlorophenyl respectively which are described and claimed in US Serial Nos. 725129, 725206 and 812730 and which are closest structurally to the compounds of formula A.

7) It is my opinion that Table II in which Ar in formulae A and B is 4-chlorophenyl shows that in compounds falling within the claims of US Patent No. 4443449 the activity decreases as the size of the group R_7 increases from methyl and ethyl to propyl whereas in the compounds of formula B falling within the claims of US Serial Nos. 725129, 725206 and 812730 the activity is maintained as the size of the corresponding group R_1 increases from methyl and ethyl to alkyl groups containing 3 and 4 carbon atoms. In my opinion this result could not have been predicted from the results obtained with the compounds of US Patent No. 4443449.

8) In my opinion Table III shows a similar situation in that for compounds of formula A increasing the size of the group R_7 to isobutyl results in a decrease in activity whereas a high level of activity is maintained as the size of the group R_1 in compounds of formula B is increased. The results shown for compounds of formula B in my opinion could not have been predicted from the results of the compounds of US Patent No. 4443449.

9) The RR_{30} values obtained for the compounds identified in the Examples of US Serial No. 725206 falling within the scope of the claims are given in Table IV. It is my opinion that Table IV shows that the compounds of US Serial No. 725206 show superior activity to the structurally closest compounds of US Patent No. 4443449 which are the compounds of formula A

in which R_7 is propyl (see Table II). The compounds in Table IV also show superior activity to the compound of formula A in Table III in which R_7 is isobutyl.

10) Based on the data set forth in Tables II to IV it is my opinion that the antidepressant activity of compounds of US Serial No. 725206 which have the formula B as set forth in these tables could not have been predicted from the results obtained with compounds of US Patent No. 4443449 of formula A in the Tables. In my opinion the compounds of formula B of US Serial No. 725206 in Table IV show unexpected superiority over compounds of US Patent No. 4443449.

11) Table V lists the RR_{30} results obtained for compounds of formula B falling within the scope of the claims of the present application which have been prepared in the laboratories of the assignees of the present application and which are not included in Tables II to IV.

Further declarant sayeth not

I, the undersigned declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States code and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

William R. Buckett

William Roger Buckett
Signed at Nottingham, England on the 30th day of March, 1987.

Table IA
[Formula A R_2 = H]

Ar	R_1	R_7	NR_3R_4	RR_{30}
4-chlorophenyl	H	H	NMe_2	61,69,36
3,4-dichlorophenyl	H	H	NMe_2	74,73
4-iodophenyl	H	H	NMe_2	79,32,61
2-naphthyl	H	H	NMe_2	61,65
4-chloro-2-fluorophenyl	H	H	NMe_2	45,49,58,52
4-chlorophenyl	H	H	pyrrolidinyl	60,71
3,4-chlorophenyl	H	H	pyrrolidinyl	70,69
2-naphthyl	H	H	pyrrolidinyl	59,79
4-chlorophenyl	H	Me	NHMe	60,72,71
4-chlorophenyl	H	Me	NH_2	54,42
4-chlorophenyl	H	Me	pyrrolidinyl	68,66
4-chlorophenyl	H	Me	NMe_2	84,81
3,4-dichlorophenyl	H	Et	NH_2	78,52,82
3,4-dichlorophenyl	H	Me	NH_2	43,49,69,64,34
3,4-dichlorophenyl	H	Et	NMe_2	69,80
3,4-dichlorophenyl	H	Me	NMe_2	79,95,45,47,68,58
4-chlorophenyl	H	Et	NHMe	57,49
3,4-dichlorophenyl	H	Et	NHMe	33,77,83,70
4-chlorophenyl	H	H	piperidino	68,58,63
4-chlorophenyl	H	H	2,5-dimethylpyrrolidinyl	60,99,75
4-chlorophenyl	H	H	4-methylpiperazinyl	68,44,70
4-chlorophenyl	H	H	1,2,3,6-tetrahydropyridyl	62,60,46,62
4-chlorophenyl	Me	H	pyrrolidinyl	64,46,70
3,4-dichlorophenyl	H	Et	pyrrolidinyl	70,81
4-chlorophenyl	H	H	N(Me)cyclopropyl	78,55,64,53

Table IB

[Formula A]

Ar	R_1	R_2	R_7	NR_3R_4	RR_{30}
4-chlorophenyl	H	H	H	NH_2	19
3,4-dichlorophenyl	H	H	H	NH_2	16,16
4-chlorophenyl	H	H	H	NHcyclopropyl	38,9
2,4-dichlorophenyl	H	H	H	NH_2	10,0
2,4-dichlorophenyl	H	H	H	NMe_2	36,29,28,51
3-chloro-4-methylphenyl	H	H	H	NH_2	22,17
3-chloro-4-methylphenyl	H	H	H	NMe_2	53,72
3-chloro-4-methylphenyl	H	H	H	pyrrolidinyl	58,51,33,37,30
4-chloro-2-fluorophenyl	H	H	H	NH_2	36,12
4-chloro-2-methylphenyl	H	H	H	NMe_2	14,17
4-chloro-3-trifluoromethylphenyl	H	H	H	NH_2	48,46
4-chloro-3-trifluoromethylphenyl	H	H	H	NMe_2	25,30,31,43
4-chlorophenyl	H	H	Et	NMe_2	45,42
4-chlorophenyl	H	H	Pr	NH_2	20,11
4-chlorophenyl	H	H	Pr	NHMe	47,34,17
4-chlorophenyl	H	H	Pr	pyrrolidinyl	1,22
4-chloro-2-fluorophenyl	H	H	Me	NH_2	61,66,38,24,35,46,21,50
4-chloro-3-trifluoromethylphenyl	H	H	Me	NH_2	56,55
4-chloro-3-trifluoromethylphenyl	H	H	Me	NMe_2	36,48
4-chlorophenyl	Me	H	Me	NH_2	26,24
4-chlorophenyl	Me	H	Me	NMe_2	36,13
4-chlorophenyl	Me	H	Me	pyrrolidinyl	0,5
4-chlorophenyl	Me	Me	H	NH_2	39,6
4-chlorophenyl	Me	Me	H	NMe_2	30,16
4-chlorophenyl	Me	Me	H	pyrrolidinyl	10
4-chlorophenyl	Me	Me	Me	NH_2	9
4-chlorophenyl	Me	Me	Me	NHMe	9
4-chlorophenyl	Me	Me	Me	NMe_2	5

Table II

[Ar = 4-chlorophenyl]

Formula A					Formula B			
R_1	R_2	R_7	NR_3R_4	RR_{30}	R_1	R_2	NR_3R_4	RR_{30}
H	H	Me	NH_2	54,42	Me	H	NH_2	70,83,58
H	H	Me	NHMe	60,72,71,70	Me	H	NHMe	67,66,59,76,66,48, 58,76,59,51,29,56
H	H	Me	NMe_2	84,81	Me	H	NMe_2	95,43,46
H	H	Me	pyrrolidinyl	68,66,55	Me	H	pyrrolidinyl	24,25,57
Me	H	H	NH_2	5				
Me	H	H	pyrrolidinyl	64,46,70				
Me	H	Me	NH_2	26,24				
Me	H	Me	NMe_2	36,13				
Me	H	Me	pyrrolidinyl	0,5				
Me	Me	H	NH_2	39,6	Me	Me	NH_2	75,51,33,72,68,16
Me	Me	H	NMe_2	30,16	Me	Me	NMe_2	9,13
Me	Me	H	pyrrolidinyl	10				
Me	Me	Me	NH_2	9				
Me	Me	Me	NHMe	9				
Me	Me	Me	NMe_2	5				
					Et	H	NH_2	52,36
H	H	Et	NHMe	57,49	Et	H	NHMe	73,53,80
H	H	Et	NMe_2	45,42	Et	H	NMe_2	79,58
H	H	Pr	NH_2	20,11	Pr	H	NH_2	81,76,78,80,83,80 75,84
H	H	Pr	NHMe	47,34,17	Pr	H	NHMe	84,75,73
H	H	Pr	pyrrolidinyl	1,22	Pr	H	NMe_2	84
Pr	H	H	pyrrolidinyl	7,26				
					i-Pr	H	NH_2	59,78
					i-Pr	H	NHMe	56,74
					Bu	H	NH_2	41,71,75
					Bu	H	NHMe	76,66,75
					Bu	H	NMe_2	76,78
					i-Bu	H	NH_2	64,61
					i-Bu	H	NHMe	79,80
					i-Bu	H	NMe_2	74,55
H	H	Ph	NH_2	5,8,9	Ph	H	NH_2	76,70,82,84
H	H	Ph	NHMe	7,22,22	Ph	H	NHMe	79,74,76,68

Table III

[Ar = 3,4-dichlorophenyl R_2 = H]

Formula A				Formula B		
R_1	R_7	NR_3R_4	RR_{30}	R_1	NR_3R_4	RR_{30}
H	Me	NH_2	43,49,69,64,34	Me	NH_2	71,72,80
				Me	NHMe	78,77,76,70
H	Me	NMe_2	76,95,48,47,68,58	Me	NMe_2	74,73,87
				Me	pyrrolidinyl	56,71,42
H	Et	NH_2	78,52,82	Et	NH_2	75,78
H	Et	NHMe	33,77,83,70,104			
H	Et	NMe_2	69,80	Et	NMe_2	84,81
H	Et	pyrrolidinyl	70,81			
				Pr	NH_2	79
				Pr	NHMe	82
				Pr	NMe_2	80
H	i-Bu	NH_2	18,29	i-Bu	NH_2	77,83
				i-Bu	NMe_2	85
H	cyclo-hexyl	NH_2	23,33	cyclo-hexyl	NH_2	78,77,63,86
				cyclo-hexyl	NHMe	83,82,83
H	cyclo-hexyl	NMe_2	17,30	cyclo-hexyl	NMe_2	56,46

Table IV

[Formula B R_2 = H]

R_1	Ar	NR_3R_4	RR_{30}
i-Bu	4-chlorophenyl	NHCHO	76,62
i-Pr	4-chlorophenyl	NHMe	56,74
s-Bu	4-chlorophenyl	NHMe	58,68
i-Bu	4-chlorophenyl	NHMe	79,80
i-Bu	2-naphthyl	NHMe	82
i-Bu	3,4-dimethylphenyl	NHMe	74,70
i-Pr	4-chlorophenyl	NH_2	59,78
s-Bu	4-chlorophenyl	NH_2	51,75
i-Bu	4-chlorophenyl	NH_2	64,61
i-Bu	phenyl	NH_2	51,51,73
i-Bu	4-chlorophenyl	NMe_2	74,55
i-Bu	phenyl	NMe_2	82,58,76,55
i-Bu	3,4-dichlorophenyl	NMe_2	85

Table V

[Formula B R_2 = H]

R_1	Ar	NR_3R_4	RR_{30}
i-Bu	phenyl	NHMe	80,78
i-Bu	3,4-dichlorophenyl	NH_2	77,83
i-Bu	2-chlorophenyl	NMe_2	75,59
i-Bu	3-chlorophenyl	NMe_2	78,82
i-Bu	4-bromophenyl	NH_2	78,78
i-Bu	4-bromophenyl	NMe_2	68,53
i-Bu	2-fluorophenyl	NH_2	75,69,45
i-Bu	2-fluorophenyl	NMe_2	67,55
i-Bu	3-fluorophenyl	NH_2	30,21,71,11,33
i-Bu	3-fluorophenyl	NMe_2	74,53,33
i-Bu	4-fluorophenyl	NH_2	62,51
i-Bu	4-fluorophenyl	NHMe	79,79
i-Bu	4-fluorophenyl	NMe_2	76,82
i-Bu	3,4-difluorophenyl	NH_2	84,83
i-Bu	3-trifluoromethylphenyl	NMe_2	80,69
i-Bu	3,4-dimethylphenyl	NMe_2	67,64
i-Bu	3,4-dimethylphenyl	NEt_2	15,13
i-Bu	4-methoxyphenyl	NH_2	59,74,66,68
i-Bu	4-methoxyphenyl	NMe_2	66,69
i-Bu	4-methoxyphenyl	N(Me)Pr	24,36
i-Bu	3-ethoxyphenyl	NMe_2	38,38
i-Bu	4-methylthiophenyl	NH_2	75,61
i-Bu	4-methylthiophenyl	NMe_2	67,76
i-Bu	2-biphenylyl	NH_2	4,7
i-Bu	3-biphenylyl	NH_2	9,7
i-Bu	4-biphenylyl	NH_2	64,69
i-Bu	4-biphenylyl	NMe_2	52,44,51
i-Bu	2-naphthyl	NH_2	82,78
i-Bu	2-naphthyl	NMe_2	82,82
i-Bu	4-biphenylyl	NEt_2	76,66
t-Bu	4-chlorophenyl	NH_2	23,19

Table V continued

R_1	Ar	NR_3R_4	RR_{30}
CH_2CMe_3	4-chlorophenyl	NH_2	83,84,83,75
CH_2CMe_3	4-chlorophenyl	NMe_2	59,85,76
CH_2CMe_3	phenyl	NH_2	11,1,18,25
CH_2CMe_3	4-methoxyphenyl	NH_2	69,57
CH_2CMe_3	4-methoxyphenyl	NMe_2	68,77
$(CH_2)_2CHMe_2$	4-chlorophenyl	NH_2	74,58
$(CH_2)_2CHMe_2$	4-chlorophenyl	NMe_2	71,33,45,68
$(CH_2)_2CHMe_2$	4-methoxyphenyl	NH_2	43,35
$(CH_2)_2CHMe_2$	phenyl	NH_2	52,54
$(CH_2)_2CHMe_2$	phenyl	NMe_2	46,76,26
$CH_2CH(Me)Et$	4-chlorophenyl	NH_2	83,78
$CH_2CH(Me)Et$	4-chlorophenyl	NHMe	79,82
$CH_2CH(Me)Et$	4-chlorophenyl	NMe_2	72,66
CH_2CHEt_2	4-chlorophenyl	NMe_2	39,53,31
CH_2CHEt_2	4-fluorophenyl	NH_2	40,9
CH_2CHEt_2	4-fluorophenyl	NHMe	38,4
CH_2CHEt_2	4-fluorophenyl	NMe_2	47,35